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RESEARCH ARTICLE

Phenological, morphological and genetic characterization of local grapevine (*Vitis labrusca* L.) genotypes grown in the Black Sea Region in Northern Turkey

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ABSTRACT

Vitis labrusca has become widely naturalized in the Black Sea region of Northern Turkey. The aim of this work was to evaluate the morphological, phenological, and genetic characteristics of *V. labrusca* accessions grown in the Black Sea region during the last three growing seasons. Local genotypes were described according to the Organisation Internationale de la Vigne et du Vin (OIV) ampelographic descriptor, including shoot length (cm), mature leaf size, bunch length and width, berry length and width, berry weight, number of berries, Total Soluble Solids (TTS) and titratable acidity. Additionally, phenological data, including bud burst, flowering, veraison and harvest date, were recorded. The accessions were characterized at the molecular level, and genetic relationships were assessed by means of Inter Simple Sequence Repeat (ISSR) markers. Using 6 ISSR primers, seventy-seven bands were obtained, of which 69 were polymorphic with a mean polymorphic rate of 88.68%. These ISSR primers produced polymorphism information content (PIC) values ranging from 0.48 to 0.5. The genetic similarity ranged from 0.08 to 0.83 among the genotypes. According to the dendrogram based on the ISSR analysis, Accessions 8 and 5 were genetically related, with a coefficient of similarity of 0.83, while Accession 3 was the most distantly related genotype, with a coefficient of similarity of 0.08. These results demonstrated that ISSR markers can be used for genetic diversity analysis among *V. labrusca* genotypes. Our results also described characteristics of new *V. labrusca* genotypes that could be valuable for future Marker-Assisted Selection (MAS) and grapevine breeding.

Keywords: Diversity; Foxy species; Inter-simple Sequence Repeat markers (ISSRs); Breeding; Polymorphism

INTRODUCTION

Grapevine (*Vitis* spp.) is an important fruit crop with a wide range of autochthonous cultivars. Most of these local grapevine genotypes belong to the species *Vitis vinifera* L., which represent a rich biodiversity important for grapevine breeding programs (Ergül et al., 2002). Over the centuries, cultivation for many years, spontaneous hybrids, vegetative reproduction, somaclonal variations, and spontaneous mutations have increased the genetic diversity in this species.

Ecological conditions are favorable for grapevine growing in almost all regions of Turkey, with the exception of the higher altitudes in Eastern Anatolia and the region along the Black Sea Coast because of heavy rainfall (Çelik et al., 2008). Due to heavy rainfall and insufficient sunshine, grape berries are not able to ripen well, fungal diseases are widespread, and low fertility is a problem in the Black Sea region (Çelik, 2004).

However, *Vitis labrusca* (L.) grape varieties and hybrids can easily be grown in the Eastern Black Sea region. While this species is native to eastern North America, it is the source of many grape cultivars and hybrid varieties, and the Rize province in the Black Sea region contains a large diversity of naturalized and open-pollinated fox grape genotypes. *V. labrusca* is more resistant to fungal diseases than other

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Vinifera species (Çelik, 2004) and serves as valuable genetic resources for breeding programs, especially for its resistance to many diseases, including phylloxera (Antcliff, 1997; Çelik, 2004). In addition, Labrusca grapes may tolerate acidic soil conditions, such as those in Northeastern Turkey (Hardie and Cirami, 1997).

V. labrusca grapes grow on trees in the forest areas of Ordu, Giresun and Rize provinces in the Black Sea coastal region and carry local names such as Isabella, black grape, aromatic grape or strawberry grape (Dırak 2009). The flavor of Isabella grapes has been described as foxy and as having special aromatic characteristics (Cangi, 1999; Çelik, 2004). Isabella grapes have a thick but easy-to-remove skin, and are therefore consumed locally as table grapes and juice or used in jam or pickles (Cangi, 1999; Çelik, 2004).

Since ampelographic descriptions are not efficient for discrimination of varieties at the clonal level and can be influenced by environmental factors and the subjectivity of the ampelographer, numerous researchers have proposed using molecular markers to identify grapevine genotypes (Akhare et al., 2008).

Genetic markers for varietal identification including Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), simple sequence repeats (SSRs), Inter SSRs (ISSRs) and Single Nucleotide Polymorphisms (SNPs). Among them, Inter Simple Sequence Repeat (ISSR) markers are relatively easy, rapid, consistent, and reliable for genetic characterization (Moreno et al., 1998). The ISSR technique was developed from a microsatellite sequence between two SSR priming sites oriented on opposite DNA strands (Moreno et al., 1998; Dhanorkar et al., 2005). ISSR markers have played an important role in identification of inter- and intra-varietal differences in grapevines (Dhanorkar et al., 2005).

Since *V. labrusca* genotypes are open-pollinated and can be fertilized by *V. vinifera*, new hybrid varieties have emerged over time, which contributes to the diversity in the region (Çelik, 2004). In recent years, both the diversity and cultivated area in this region has been decreasing due to ecological conditions (Dırak, 2009). Even though labrusca grapes have been available to growers for many years, it is a poorly explored cultivar and cultivated only in the northern part of Turkey. In order to establish new commercial vines and to establish vineyards in this region, it is important to document the phenological, morphological and quality characteristics of plants from this species.

The aim of this study was to evaluate the ampelographic characteristics of local labrusca grape genotypes grown in the Rize province in the northeastern part of Turkey using a combination of ampelographic description, with OIV and IBPGR descriptors, and molecular ISSR markers. This is especially important for ensuring the protection of valuable grape genetic resources in the region, which could provide beneficial alleles for plant breeding and improvement.

MATERIAL AND METHODS

Plant material

All accessions were grown in an experimental vineyard at the Research and Implementation Center of Recep Tayyip Erdogan University (RTE University) in the province of Rize in the Black Sea region during three consecutive vegetation periods (2016, 2017 and 2018). Eleven local labrusca accessions were collected based on their different pomological and phenological characteristics from different locations in Rize and were preserved at the Research and Implementation Center of RTE University. Accessions were grown and evaluated to determine their ampelographic and molecular relationships All isolates were derived from backyard gardens.

The experimental area consisted of six rows with 5 plants each, spaced 2.0 m between rows and 2.0 m within rows. The vines were 10 years old, grown with their own roots, and cultivated under the same growing conditions. Over all seasons, the vines were cane pruned, retaining six to eight nodes on each of the 12 to 14 canes maintained per vine. The grapevines were trained on a wall training system and planted in acidic soil conditions.

Ampelographic characterization

The International Board for Plant Genetic Resources (IBPGR) publication Grape Descriptors (The International Board for Plant Genetic Resources, 1983) and the revised Descriptors for Grapevine (*Vitis spp.*) (International Organization of Vine and Wine 1997) were used to ampelographically characterize the selections. Eleven OIV descriptors were chosen to present basic information on naturalized labrusca grapevines. Morphological descriptions were assessed for 5 previously selected and identified vines per genotype during three vegetation seasons from 2016 to 2018.

Ten average shoots per variety were chosen for analysis. The mature leaf descriptions were recorded between berry set and véraison (beginning of berry maturity) on leaves above the cluster within the medium third of the shoot. The clusters were measured when matured. The berry characteristics were obtained at ripening for berries located in the middle of the bunches. The evaluations were made from budburst to harvesting time as indicated by the OIV.

Phenological characteristics were evaluated weekly and expressed in days after pruning (DAP) for the 2016, 2017 and 2018 seasons. Budburst, blooming and véraison were evaluated when 50% of the plants showed these physiological responses. Ripening dates were determined according to total soluble solids (TSS) and recorded in °Brix, using an digital refractometer. Titratable acidity was determined following the procedure described by Tyl and Sadler (2017). Although a level of 16 °Brix is generally considered necessary to satisfy maturity (CODEX Standard 255, 2007; OIV, 2008; United Nations Economic Commission for Europe, 2010), harvesting time varied by variety.

DNA extraction and ISSR analysis

Eleven cultivars were analyzed by PCR using ISSR primers. Cultivars 'Sultana' and 'Alphonse Lavallée' of *V. vinifera* were used as reference varieties. Genomic DNA was isolated from 2 grams of young leaves following the procedure described by Doyle and Doyle (1987). Genomic DNA was checked for its integrity and quantified on agarose gels.

PCR reactions were carried out in a 25-µl reaction mix containing 50 ng of template DNA, 0.3 U of Taq DNA polymerase (Fermentas), 2 mM $MgCl_2$, 200 µM of each dNTP (Fermentas) and 10 pmol each primer. PCR reaction was set using the following program: Initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 45 s and extension at 72 °C for 2 min with a final extension at 72 °C for 7 min. PCR products were visualized on 1% agarose gel (Fermentas) stained with ethidium bromide (0.5 µg/ml). BioOneD software (Fermentas, USA) was used to estimate the allele sizes by comparison with the standard DNA molecular weight markers. Resulting bands were recorded as present (1) or absent (0). Jaccard similarity matrix was constructed by NTSYSpc 2.1 software. The unweighted pair group method with arithmetic mean (UPGMA) dendrogram was constructed using NTSYS pc 2.1 software (Rohlf, 2005). For primers, polymorphism information content (PIC) values were calculated according to the formula: $PIC = 1 - \Sigma(P_{ij})2$, where P_{ij} is the frequency of the i_{th} pattern revealed by the j_{th} primer summed across all patterns revealed by the primers (Botstein et al. 1980).

Statistical analysis

A complete randomized block design with three replicates (consisting of five vines) was established. Data were separately evaluated for each vine by analysis of variance (ANOVA), and treatment means were separated by Least Significant Differences (LSD) test at $P < 0.05$. Analysis was performed with the JMP program (v 8.00, SAS Institute Inc., NC 27513-2414, USA).

RESULTS AND DISCUSSION

Local labrusca accessions that have been cultivated as landraces in the northern region of Turkey were collected as rooted plants (which were maintained) and transferred to a vineyard at the Experimental Research Area of RTE University. The eleven accessions constituted our ampelographic collection and were evaluated using a combination of OIV ampelographic descriptors, phenological and morphological traits, berry oenological traits, and molecular genetics using six ISSR markers. These accessions were presented in Fig. 1.

Phenological data

Phenological data such as budburst, blooming, véraison, and harvest dates were obtained for 2016, 2017 and 2018 are presented in Table 1. The onset of bud burst varied from 16 March (Accession 1) to 20 March (Accessions 3, 4, 7, and 11) for 2016 and from 3 March (Accessions 1 and 2) to 8 March (Accessions 3, 4, 9, 10, and 11) for 2017 and from 5 March (Acession 1) to 13 March (Acessions 11) for 2018. Among the accessions, blooming dates occurred between 1 June (Accessions 1 and 2) and 12 June (Accessions 9, 10, 11) during 2016 and between 27 May (Accessions 1 and 2) and 8 June (Accessions 9, 10, 11) during 2017 and between 30 May (Accessions 1, 2, and 3) and 10 June (Accessions 10 and 11) during 2018 (Table 1). Accessions 1 and 2 were the first to budburst, while Accessions 9, 10 and 11 were the last during 2016, 2017 and 2018. Accessions 1 and 2 were the first to bloom for all three years (Table 1). Similarly, Labrusca grapes were reported to bloom from May to June (Çelik et al., 2004).

Accessions 3 and 4 were the first to reach véraison in both 2016 and 2017 (28 and 31 July, respectively, for 2016 and 1 August in both accessions for 2017). Accession 4 was the first to reach véraison while Accession 8 was the last for 2018. For 2016, the earliest ripening was recorded on 4 September (Accession 3), whereas the latest ripening was observed on 19 September (Accessions 6, 8, and 11) (Table 1). In the second year of the study, the earliest ripening was observed on 28 August (again Accession 3), while the latest ripening dates were 20 September (Accessions 8, 10, and 11). In 2018, the earliest ripening was observed on 28 August (Accessions 3and 4), while the latest ripening dates were 20 September (Accession 11). According to the phenological data presented in Table 1, there was a difference of 15-20 days in terms of ripening dates among the accessions. In another study of fox grape cultivars in Samsun province, bud burst was as late as April, full-bloom occurred between May and June, veraison began in the first week of August, and harvest dates were in September (Köse, 2014).

Fig 1. Images of shoot, leaf and fruit characteristics of labrusca accessions.

Table 1: Phenological observations of eleven *V. labrusca* accessions from the Black Sea region of Turkey.

Accession	Budburst			Flowering			Veraison			Ripening		
	2016	2017	2018	2016	2017	2018	2016	2017	2018	2016	2017	2018
1	16 March	3 March	5 March	1 June	27 May	30 May	8 Aug.	5 Aug.	6 Aug.	6 Sept.	31 Aug.	7 Sept.
2	18 March	3 March	6 March	1 June	27 May	30 May	11 Aug.	10 Aug.	12 Aug.	7 Sept.	2 Sept.	4 Sept.
3	20 March	8 March	11 March	3 June	29 May	30 May.	28 Aug.	1 Aug.	1 Aug.	4 Sept.	28 Aug.	28 Aug.
4	20 March	8 March	8 March	5 June	30 May	28 May	31 july	1 Aug.	1 Aug.	5 Sept.	1 Sept.	28 Aug.
5	18 March	4 March	10 March	7 June	1 June	5 June	5 Aug.	10 Aug.	3 Aug.	8 Sept.	15 Sept.	4 Sept.
6	19 March	4 March	11 March	8 June	1 June	5 June	16 Aug.	16 Aug.	4 Aug.	19 Sept.	17 Sept.	4 Sept.
7	20 March	7 March	9 March	10 June	4 June	5 June	15 Aug.	14 Aug.	15 Aug.	18 Sept.	16 Sept.	13 Sept.
8	17 March	4 March	12 March	11 June	5 June	9 June	13 Aug.	17 Aug.	19 Aug.	19 Sept.	20 Sept.	11 Sept.
9	19 March	8 March	11 March	12 June	8 June	6 June	12 Aug.	15 Aug.	8 Aug.	16 Sept.	16 Sept.	10 Sept.
10	18 March	8 March	12 March	12 June	8 June	10 June	12 Aug.	15 Aug.	11 Aug.	18 Sept.	20 Sept.	13 Sept.
11	20 March	8 March	13 March	12 June	8 June	10 June	13 Aug.	17 Aug.	15 Aug.	19 Sept.	20 Sept.	20 Sept.

Table 2: The number of days between phenological stages in *V. labrusca* accessions from the Black Sea region of Turkey.

Accession	Budburst- Flowering			Flowering- Veraison			Veraison- Ripening		
	(2016)	(2017)	(2018)	(2016)	(2017)	(2018)	(2016)	(2017)	(2018)
1	77	85	72	68	70	74	29	26	32
2	75	85	71	71	75	72	27	23	24
3	75	82	71	55	64	61	38	27	27
4	77	83	72	56	63	63	36	31	27
5	81	89	75	59	70	66	34	36	30
6	81	89	76	69	76	73	34	32	32
7	82	89	77	66	71	70	34	33	28
8	86	93	94	63	73	70	37	34	23
9	85	92	86	61	68	62	35	32	32
10	86	92	82	72	76	61	38	33	33
11	85	91	87	69	75	65	34	29	35

The number of days from budburst to blooming, from blooming to véraison, and from véraison to ripening are presented in Table 2. The number of days from budburst to blooming varied from 75-86 days for 2016 and from 82-89 days for 2017, from 71-94 days for 2018. The longest transition from budburst to blooming was for Accession 8 (93 days in 2017). The number of days from blooming to véraison varied from 55-71 days for 2016 and from 63-76 days for 2017 and from 61-74 days for 2018. The longest transition from blooming to véraison was for Accessions 6 and 10 (76 days in 2017). The number of days from véraison to ripening ranged between 27-38 days for 2016 and 23-36 days for 2017 and from 23-35 days for 2018. The longest transition from véraison to ripening was for Accessions 3 and 10 (38 days in 2016).

Evaluation of morphological and pomological characteristics

Average values of the morphological parameters of the eleven local labrusca accessions are given in Table 3. According to our results, there were significant differences between shoot and leaf lengths among the accessions ($P < = 0.05$). Accession 3 had the longest shoot (139.04 cm), while Accession 5 had the shortest shoot (113.94 cm) (Table 3).

The maximum leaf width was found in Accession 3 (26.32 cm), whereas the minimum leaf width was obtained in Accessions 5 (17.34 cm) and 10 (17.45 cm). The highest values for leaf length were found in Accession 4 (18.11 cm) and Accession 3 (18.08 cm), whereas the lowest values were found in Accessions 5 (13.77cm) and 10 (13.64cm). In accordance with our results, Celik et al. (2008) reported that the width of the mature leaf was between 14.1 and 20.8 cm and that the mature leaf length varied between 12.7 and 20.6 cm in *V. labrusca* grapevines.

In table grape varieties (*V. vinifera*), characteristics of the cluster and the chemical and physical properties of the grape are among the important parameters affecting quality and consumer preferences (Aydın, 2009; Kamiloğlu, 2013). In this study, there were differences (at the 5% significance level) among the eleven accessions in terms of cluster weight, cluster width and cluster size. Accession 4 had the highest cluster weight (133.99 g), whereas Accession 6 had the lowest cluster weights were found in Type 6 (79.71) and Type 1 (83.15) (Table 3). In Labrusca grapes, Sabır (2008) found the average cluster weight to be 235.4 g, while Dırak (2009) reported the average cluster weight as 109.70 g.

Accession 4 had the highest value for cluster width (84.25 mm), correlating with its highest weight, while Accession 1 had the lowest cluster width (61.01 mm). The highest cluster length was 135.39 mm for Accession

Table 3: Average values for 14 Descriptors in eleven *V. labrusca* accessions

OIV Code Number	Trait description	Accession 1	Accession 2	Accession 3	Accession 4	Accession 5	Accession 6	Accession 7	Accession 8	Accession 9	Accession 10	Accession 11
	Shoot length (cm)	126,90^{bc}	123,97^{bcd}	139,04^{a}	133,84^{ab}	113,94^{d}	117,32^{cd}	125,08^{bcd}	118,63^{cd}	122,86^{bcd}	118,65^{cd}	127,02^{bc}
-	Mature leaf width (cm) (A)	21,90^{cd}	18,36 ^{f}g	26,32^{a}	26,24^{b}	17,34 g	20,55^{de}	22,02^{cd}	19,70^{ef}	22,79^{bc}	17,45g	22,04^{cd}
-	Mature leaf length (cm) (B)	16,89^{bc}	15,51^{d}	18,08^{a}	18,11^{a}	13,77^{e}	15,67^{d}	17,72^{ab}	14,96^{d}	15,76^{cd}	13,64^{e}	16.90^{bc}
OIV 065	Mature leaf size (AxB)	369,99^{b}	285,35^{d}	476,74^{a}	439,24^{a}	238,83^{e}	321,36^{cd}	390,08^{b}	294,99^{d}	359,01^{bc}	238,01^{e}	372,48^{b}
OIV 202	Bunch length (mm) (E)	111,34^{bcd}	110,62^{bcd}	107,94^{cd}	119,61^{abc}	100,64^{d}	104,65^{cd}	127,03^{ab}	119,68^{abc}	135,39^{a}	110,99^{bcd}	111,29^{bcd}
OIV 203	Bunch width (mm) (D)	61,02^{d}	61,65^{cd}	73,71^{b}	84,25^{a}	70,21^{b}	68,06^{bc}	69,51^{b}	73,67^{b}	71,00^{b}	61,60^{cd}	68,20^{bc}
OIV 205	Bunch: number of berries	32,66^{c}	30,61^{c}	34,66^{bc}	43,12^{a}	29,76^{c}	30,74^{c}	35,01^{bc}	34,75^{bc}	38,59^{ab}	35,87^{bc}	38,50^{ab}
OIV 502	Bunch: weight of a single bunch (g)	83,15^{e}	92,43^{de}	102,38^{cd}	133,99^{a}	86,79^{de}	79,71^{e}	113,79^{bc}	113,30^{bc}	127,83^{ab}	113,70^{bc}	113,29^{bc}
OIV 221	Berry width (mm) (F)	16,08^{b}	17,09^{ab}	16,81^{b}	32,58^{a}	16,04^{b}	16,40^{b}	17,08^{ab}	16,68^{b}	17,51^{ab}	16.80^{b}	16.81^{b}
OIV 220	Berry length (mm) (G)	16,45^{c}	18,31^{a}	17,31^{b}	17,29^{b}	16,58^{c}	16,81^{bc}	18,26^{a}	18,01^{a}	18,31^{a}	17.30^{b}	17.29^{b}
	Berry size (F x G)	266,36^{b}	313,32^{ab}	291,36^{ab}	561,12^{a}	266,43^{b}	275,82^{b}	312,19^{ab}	300,72^{ab}	321,36^{ab}	290,64^{ab}	290,64^{ab}
OIV 503	Berry: single berry weight (g)	3,05^{cde}	3,42^{ab}	3,25^{bcd}	3,35^{abc}	2,86^{e}	2,99^{de}	3,34^{abc}	3,20^{bcd}	3,59^{a}	3,41^{ab}	3,40^{ab}
OIV 505	TTS (^{o}brix)	15,73^{cd}	16,93^{ab}	14,83^{ef}	15,67^{cde}	17,8^{a}	16,52^{bc}	14,70^{f}	15,13^{def}	16,30^{bc}	15,70^{cd}	14,80^{ef}
OIV 506	Titratable acidity (%)	0,48^{b}	0,55^{ab}	0,46^{b}	0,46^{b}	0,62^{ab}	0,46^{b}	0,62^{ab}	0,65^{a}	0,58^{ab}	0,47^{b}	0,46^{b}

[1]Data within each column represent the means of the two harvest seasons per genotype. Means followed by same letters within columns are not different ($p < 0.05$)

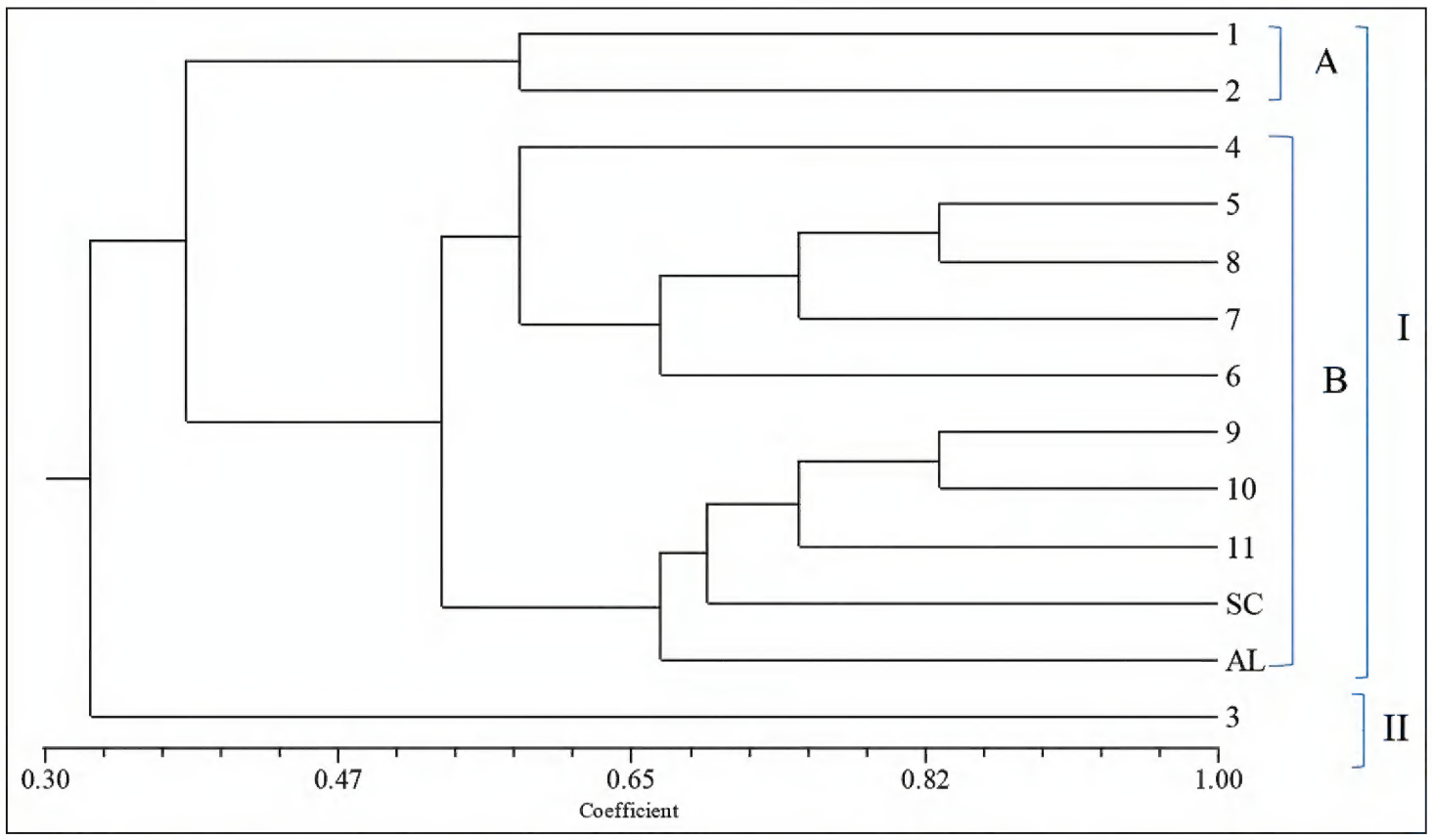

Fig 2. UPGMA dendrogram of the leven *V. labrusca* accessions and two *V. vinifera* cultivars on the basis of ISSR data.

9, whereas the shortest cluster length was 100.64 mm for Accession 5 (Table 3). Sabır (2008) reported that the average cluster width was 9.66 cm and the average cluster length was 14.46 cm, while Dırak (2009) reported a cluster width of 69.22 mm and a cluster length of 107.42 mm.

The sizes of the berries in the cluster are important in terms of quality and yield. When the properties of the cluster and berries of the accessions were examined, there were differences between the genotypes at the 5% significance level in terms of berry weight, berry width, berry size, bead number and total soluble solids (TSS) content. Among the accessions in this study, Accession 9 had the highest value for berry weight (3.59 g) while Accession 5 had the lowest value for berry weight (2.86 g) (Table 3). The results obtained in our study were comparable to that of Cangi et al. (2006) and Sabır et al. (2008). Cangi et al. (2006) reported that the berry weight in 11 local foxy grapes selected from the Ordu-Giresun regions varied between 2.19 and 3.57 g. Sabır (2008) reported a berry weight of Isabella hybrid as 3.95 g. Çelik et al. (2008) reported that the berry weights of 18 foxy genotypes varied between 1.49 and 5.28 g. Accession 4 produced the widest berry (38.52 cm), while Accession 5 (16.04 mm), Accession 1 (16.08 mm), Accession 6 (16.40 mm) and Accession 3 (16.81 mm) produced the narrowest berries (Table 3). Accessions with the largest berry were Accessions 2 and 9 (18.31 mm), Accession 7 (18.26 mm) and Accession 8 (18.01 mm), while Accessions with the shortest berry were Accession 1 (16.45 mm) and Accession 5 (16.58 mm) (Table 3).

Among the accessions, Accession 4 had the highest number of berries per bunch (43.12), while the lowest numbers of berries were found in Accession 5 (29.76), Accession 2 (30.61), Accession 6 (30.74) and Accession 1 (32.66) (Table 3). Similarly, most of the investigated fox grape selections had more than 20 and less than 50 berries in a cluster (Çelik, 2004; Dırak, 2009).

Total soluble solids (TSS) content ranged from 14.70 to 17.80 °Brix across both harvest seasons (Table 3). Accession 5 distinguished itself by presenting the highest values for TSS (17.8 °Brix), while °Brix 7 had the lowest values (14.70 °Brix). These values are in accordance with the 14.5-18 °Brix found by Cangi et al. (2006), and the 12-22.2 °Brix observed by Çelik et al. (2008). Dırak (2009) reported that the TSS content in Isabella was 21.93% in Tekirdağ. Kose (2014) determined a TSS content of 21.4% in *V. labrusca* grown in Samsun province. This study found similar TSS content results to the studies conducted by Cangi et al. (2006), Sabır (2008), and Çelik et al. (2008). In the studies conducted by Dırak (2009) and Kose (2014), the higher TSS content may be related to different ecological conditions.

The highest titratable acidity of grape juice was found in Accession 8 (0.65%), while the values were found in Accessions 3, 4, 6 (0.46%) and Accession 1 (0.48%) (Table 3). Cangi et al. (2006) reported that the acidity ranged between 4.7 to 6.8 g/L in 11 local foxy grape selections. Similarly, Sabır (2008) determined an acidity of 5.40 g/L. Differences between acidity values may be due to genetics and climactic conditions of the region in which the experiments were conducted.

ISSR Polymorphisms

There are few primers for ISSR markers that are efficient for characterization of grape varieties (Herrera et al. 2002; Sabir et al. 2008). Of these, six primers were chosen, based on

their ability in previous studies to distinguish closely related cultivars (Herrera et al., 2002; Sabır et al., 2009). Using 6 ISSR primers, seventy-seven bands were obtained through PCR amplification from the 11 Labrusca accessions. Sixty-nine of these markers were polymorphic, with a mean polymorphic rate of 88.68% (Table 4). The total number of bands per primer ranged from 10 to 17, with an average of 12.83. The size of the amplified fragments varied from 310 bp to 2100 bp (Table 4). The total number of bands per primer ranged from 10 to 17, with an average of 12.83. In the previous studies, the total number of bands varied from 2 to 13 per ISSR primer (Moreno et al., 1998; Herrera et al., 2002; Sabir et al., 2008). The size of the amplified fragments varied from 310 bp to 2100 bp (Table 4). This interval was comparable to that obtained by Moreno et al. (1998) and by Sabır et al. (2009) who reported between 300 bp and 2500 bp for different grape varieties.

The number of polymorphic bands per primer ranged from 7 to 17, with an average of 11.5. One primer, UBC885, resulted in 100% polymorphism, while the lowest rate was obtained from UBC834 (70%) (Table 4). The primer (AG)8YT produced only 7 bands, while (AG)8C and BDB(CA)7 amplified the highest number (17) of bands. Similarly, previous genetic assessment studies of *V. vinifera, V. labrusca* and *V. rotundifolia* cultivars have obtained polymorphism rates ranging between 60 and 100% with ISSR primers (Dhanorkar et al. 2005; Wu et al. 2006). While it has been reported that GA and GT repeats are most highly represented in the *Vitis* genome, in our study primers detecting AG nucleotide repeats were more polymorphic.

Table 4: ISSR marker analysis of 11 V. labrusca accessions from the Black Sea region of Turkey. The number of total bands (NTB), number of polymorphic bands (NPB), polymorphism rate (PR), polymorphism information content (PIC) of the primers, and the product sizes of each marker.

Primers	NTB	NBP	PR%	PIC	Product size
UBC808 (AG) 8C	17	15	93,75	0.4995	310-1900 bp
UBC826 (AC) 8C	11	10	90,90	0.4989	390-1600 bp
UBC834 (AG) 8YT	10	7	70	0.4844	600-2100 bp
UBC885 BHB (GA) 7	11	11	100	0.5	395-1450 bp
UBC887 DVD (TC) 7	12	10	83,33	0.4959	450-1500 bp
UBC888 BDB (CA) 7	17	16	94,11	0.4995	370-1900 bp
Total	78	69			
Mean	12.83	11.5	88.68		

A dendrogram was constructed by UPGMA using a distance matrix and shows clustering of the selections based on the ISSR markers (Fig. 2). The dendrogram analysis identified two major groups (Fig. 2), constituted by several subgroups. Two of the clusters contained individuals from both Rize and Samsun provinces, while one cluster contained both Labrusca accessions and the reference cultivars Sultana and Alphonse Lavallee. One outlier, Accession 3, formed its own subgroup, appearing to be the most divergent genotype. In the first branch of first group of cluster (A), Accessions 1 and 2 from Rize province are more distant from the rest of genotypes. Interestingly, in the second branch of first group of cluster (B), the two reference cultivars, Sultana and Alphonse Lavalle, were found to be genetically related with the local Labrusca accessions 9, 10, and 11, which may be due to open pollination of Labrusca grapes with *V. vinifera* varieties in the region over time.

The genetic similarity ranged from 0.08 to 0.83 in the ISSR analysis. According to the dendrogram, Accessions 8 and 5 were genetically closely related, as Accessions 9 and 10, with both sets having coefficient of similarities of 0.83. Accession 3 was the most distant genotype, with a coefficient of similarity to Accessions 10 and 11 of 0.16 (Table 5).

Table 5: Jaccard's distance index generated on using ISSR data from 11 local Labrusca accessions and two reference genotypes.

Accessions	1	2	3	4	5	6	7	8	9	10	11	S	AL
1	1												
2	0.583	1											
3	0.083	0.25	1										
4	0.083	0.417	0.5	1									
5	0.417	0.417	0.333	0.667	1								
6	0.25	0.25	0.583	0.5	0.667	1							
7	0.25	0.583	0.583	0.667	0.667	0.583	1						
8	0.417	0.417	0.417	0.5	0.833	0.75	0.833	1					
9	0.333	0.333	0.333	0.417	0.667	0.667	0.583	0.75	1				
10	0.5	0.5	0.167	0.333	0.583	0.5	0.417	0.583	0.833	1			
11	0.5	0.5	0.167	0.333	0.5	0.417	0.417	0.5	0.667	0.833	1		
S	0.417	0.417	0.25	0.417	0.667	0.667	0.5	0.667	0.667	0.75	0.667	1	
AL	0.333	0.333	0.25	0.333	0.583	0.583	0.583	0.75	0.75	0.667	0.583	0.667	1

CONCLUSION

In recent years, a decrease in total vineyard area in Turkey has been observed for various reasons. This situation raises the possibility of loss of grape genetic resources that have not yet been defined (Çelik et al., 2013). In this study, eleven local Labrusca accessions have been evaluated using a combination of OIV ampelographic descriptors, phenological and morphological traits, berry oenological traits and molecular genetics using Inter Simple Sequence Repeats (ISSRs) markers. We detected high genetic variation among the Labrusca genotypes using 6 ISSR primers, which validated that the ISSR technique is a powerful genetic tool in grapevine germplasm characterization. In addition, these local Labrusca genotypes may offer a genetic resource for future breeding programs.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Author's contributions

Birsen Çakır Aydemir and Kezban Yazıcı designed the eperiments, discussed the results, and wrote the original draft. Burcu Göksu participated in the development of the experiment.

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